# Biliary Excretion of Copper, Manganese and Zinc in Humans

AUTHOR(S):

SEKIYA, TSUKASA; TANIMURA, HIROSHI; HIKASA, YORINORI

CITATION:

SEKIYA, TSUKASA ...[et al]. Biliary Excretion of Copper, Manganese and Zinc in Humans. 日本外科宝函 1982, 51(6): 862-881

ISSUE DATE:

1982-11-01

URL:

http://hdl.handle.net/2433/208989

Arch Jpn Chir 51(6), 862~881, Nov., 1982

# Biliary Excretion of Copper, Manganese and Zinc in Humans

TSUKASA SEKIYA, HIROSHI TANIMURA and YORINORI HIKASA

The 2nd Department of Surgery, Faculty of Medicine, Kyoto University
(Director: Prof. Dr. YORINORI HIKASA)
Received for Publication, September 7, 1982.

## Introduction

Copper, manganese and zinc, though found in only trace amounts, are essential elements which play an important role in human growth and development[53]; Wilson's disease, Menkes' hair kinky syndrome and accidental intoxication are well known disorders associated with these elements. Zinc and/or copper deficiencies during total parenteral nutrition have been frequently reported[26,27], and the role of these elements in cancer has been studied[41]. As research of the trace elements progresses, more diseases associated with them may be found and, thus, the elucidation of their metabolism is of great importance.

Interestingly, these elements are mainly excreted into the feces, compared to other major ions which are excreted into the urine[53,29]. From previous animal experiments, bile in particular, has been found to be an important pathway for copper and manganese. However, in humans, investigation of the biliary excretion of trace elements has been limited for several reasons, such as the lack of proper analytical techniques and the difficulty of handling samples because their concentrations in biological samples are too low and the samples are easily contaminated. Many previous investigators have approached these problems by the tracer method, but it is not adequate for observing the natural excretion of endogenous trace elements though it is suitable for the exogenous ones.

In order to further understand the biliary excretion of copper, manganese and zinc in humans, these elements, collected continuously from the bile of patients with biliary fistula for cholelithiasis, were analysed by a new technique using flameless atomic absorption spectrometry, which is sensitive enough and requires only a small amount of sample[42].

Biliary fistulas were made using two kinds of choledocal T tubes. One is a conventional T tube made of silicon which prevents contamination of the collected sample, and the other is a T tube with a bulging cuff which prevents bile flow into duodenum. The enterohepatic circulation is an important problem in the study of the biliary excretion. By using this tube, it was easy to collect more complete bile samples, and that the obtained data were easily interpreted. However, unfortunately, as this T tube is made of rubber, there is some contamination of the zinc.

In this study, the individual differences and diurnal deviations posed considerable problems,

Key word: Bile, Trace elements, Total parenteral nutrition, Gallstone.
索引語：胆汁，微量元素，完全静脈栄養，胆石.
Present address: The 2nd Department of Surgery, Faculty of Medicine, Kyoto University. 54 Kawara-cho, Shogoin, Sakyo-ku, Kyoto 606 Japan.

thus other bile components in addition to trace elements were simultaneously determined and the comprehensive results from many cases were noted to avoid experimental errors.

Furthermore, based on these results, the dosage of trace elements in the total parenteral nutrition and their role in gallstone formation were considered.

## Materials and Methods

*Patients*

Fourteen patients who had been operatively inserted with choledocal T tubes after gallstone extirpation were admitted to Kyoto University Hospital. The patients were divided into two groups. The first group with conventional T tubes, consisted of three females and four males; their ages ranged from 46–73 yr (body weight, 40–68 kg). Bile was collected seven or 21 days after the operation (Table 1). The second group, with the special T tubes with the cuffs, consisted of three females and four males; their ages ranged from 46–67 yr and their weight from 44 to 60 kg (Table 2). Bile collection was started 13 or 35 days after the operation. Except for Case 14, it was the first time for these patients to undergo an operation for cholelithiasis; in all cases the gallbladder was extirpated. The gallstones were classified into three types: cholesterol, mixed stone consisting of cholesterol and bilirubin, and pigment stone[21]. In Case 14, the gallstone had originated from the choledochus but in other cases, they might have originated from the gallbladder and migrated into the choledochus.

The biliary tracts of the patients were examined before starting the study by postoperative cholangiography. All biliary passages were smooth and no residual stones were observed; the clinical data were within normal ranges and neither proteinuria nor renal dysfunction was noted. The patients usually took breakfast at 8: 30 AM and lunch at 12 noon.

*Sample Collection*

In the first group, bile, plasma and urine were collected to study the influence of the administration of "Trace Elements Solution®" (Midori Juji Co. L. T. D. Osaka. Japan) or "Trace Minerals Solution®" (Morishita Pharmaceutical Co. L. T. D. Osaka, Japan) made for total parenteral nutrition. One ampoule of the former solution contains 2.1 mg of $CuSO_4 \cdot 5H_2O$,

**Table 1.** Patients in the First Group.

| Case | Age | Sex | Body Weight (Kg) | Type of Stones | Postoperative Days | Bile Collection Schedule | |
|---|---|---|---|---|---|---|---|
| | | | | | | 1st | 2nd |
| 1 | 57 | male | 54 | Cholesterol | 10 - 11 | * | T.E. 5A. |
| 2 | 47 | male | 68 | Mixed | 8 - 9 | * | T.M. 5A. |
| 3 | 48 | male | 55 | Cholesterol | 8 - 9 | * | T.M. 5A. |
| 4 | 46 | female | 67 | Mixed | 15 - 16 | T.E. 5A. | * |
| 5 | 56 | male | 58 | Cholesterol | 20 - 21 | T.E. 5A. | * |
| 6 | 73 | female | 40 | Mixed | 7 | T.E. 5A. | * |
| 7 | 48 | female | 58 | Cholesterol | 7 | T.E. 3A. | * |

* Bile samples were collected without the infusion of the trace elements solution.
T.E.: Trace Elements Solution®. T.M.: Trace Minerals Solution®.

**Table 2.** Patients in the Second Group.

| Case | Age | Sex | Body Weight (Kg) | Type of Stones | Postoperative Days | Bile Collection Schedule | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 1st | 2nd | 3rd |
| 8 | 61 | female | 49 | Mixed | 27 - 28 | * | * | |
| 9 | 61 | male | 54 | Mixed | 19 - 20 | * | * | |
| 10 | 67 | male | 60 | Cholesterol | 13 - 15 | * | Cu 1 mg | * |
| 11 | 46 | female | 44 | Mixed | 18 - 19 | * | T.E. 1A. | |
| 12 | 59 | male | 50 | Mixed | 17 - 18 | * | Mn 1mg | |
| 13 | 64 | female | 45 | Mixed | 35 | * | | |
| 14 | 67 | male | 58 | Bilirubin | 15 | * | | |

* T.E.. see Table 1. In all cases bile samples were collected every 20 minutes from 9 AM to 3 PM.

4.8 mg of $MnCl_2 \cdot 4H_2O$, 5.9 mg of $ZnSO_4 \cdot 7H_2O$ and 1.6 mg of $CoCl_2 \cdot 6H_2O$. That of the latter contains 0.85 mg of $CuCl_2 \cdot 4H_2O$, 5.8 mg of $ZnSO_4 \cdot 7H_2O$, 7.9 mg of $MnCl_2 \cdot 4H_2O$ and 0.17 mg of $KI_2$ (Table 3). One to five ampoules of either solution was administered with 500 ml of 5% glucose dripping intravenously from 9 AM to 11 AM. Bile samples were collected hourly from 8 AM to 3 PM for one or two days. Plasma samples were obtained from 9 AM to 3 PM every two hours and urine samples in Cases 3 to 5 were continuously taken every one or two hours. To avoid contamination during these procedures, bile and urine samples were directly collected in polypropylene tubes and stored at —20°C until analysis.

In the second group, to characterize the biliary excretion of endogenous trace elements, other components in bile (total bilirubin, total bile acids, total protein, magnesium and calcium) were simultaneously determined along with other trace elements. Bile samples were collected from the special T tubes preventing the bile flow into duodenum. Details of the procedure are as follows. Firstly, before starting bile collection, the external biliary fistulas were closed for three days so as to restore the impaired enterohepatic circulation which resulted from the loss of bile after the operation. Then bile collection was begun by opening the fistulas soon after the cuffs were expanded by the infusion of about 1 ml of saline at 9 AM. Bile was continuously collected every 20 minutes from 9 AM to 3 PM in polypropylene tubes which were sealed by black paper to prevent light-induced bilirubin degeneration. Total bilirubin was measured in less than one hour and the residual bile sample was dividing into two tubes, for the determination of protein and other bile

**Table 3.** Copper, Manganese and Zinc Volumes in the Trace Elements Solution.

| | | Cu (mg) | Mn (mg) | Zn (mg) |
|---|---|---|---|---|
| Trace Elements Solution® | 1A. | 0.5 | 1.3 | 1.0 |
| | 3A. | 1.6 | 3.9 | 3.0 |
| | 5A. | 2.7 | 6.7 | 5.0 |
| Trace Minerals Solution® | 1A. | 0.3 | 2.2 | 1.3 |
| | 5A. | 1.6 | 11.0 | 6.5 |

components, and stored. After collecting the bile, external fistulas were again closed and cuffs were deflated by removing the saline, for the normalization of the enterohepatic circulation. The same procedures were repeated in some patients for two or three days. In Cases 10 to 12, mixed or single trace elements were infused to confirm their interaction in biliary excretion.

The schedule of bile collection is shown in Tables 1 and 2.

*Analysing Method*

Copper, manganese and zinc were determined using flameless atomic absorption spectrometry (Shimadzu AA 640–13 model, Shimadzu GFA-2 model, Kyoto, Japan) by the direct dilution method with pure water for bile and by the wet digestion method with concentrated $HNO_3$ and $HClO_4$ for plasma and urine[42]. Magnesium and calcium were determined by flame atomic absorption spectrometry as is used in clinical examination. Total bilirubin in bile was assayed by the MICHAELSSON method[35] with modification and total bile acids were assayed by the enzymic method[48]. Total protein in bile was determined by the Coomassie Brilliant Blue method[7]. During all analytical procedures, great care was taken to avoid contamination while handling the samples.

## Results

From the first group, several interesting findings were obtained. Firstly, the concentrations of copper, manganese and zinc in the bile of the seven patients showed a wide range compared with those in plasma. The values in the bile just before the infusion of trace element solutions were: copper, 4–140 $\mu$g/dl; manganese, 1.3–41.5 $\mu$g/dl; and zinc, 2.6–28.2 $\mu$g/dl. The concentrations in the plasma of these patients obtained at 9 AM were: copper, 72 to 103 $\mu$g/dl; manganese, 0.7 to 1.3 $\mu$g/dl; and zinc, 84 to 150 $\mu$g/dl. This large individual deviation in the bile demonstrates that the bile is an excretory media for these elements.

Next, the subsequent changes in these concentrations in the bile and plasma with the administration of trace elements were observed for seven hours. Although in rats, it has been reported that a large part of the injected copper is excreted within short time[10,38], in this study, no remarkable increase in copper concentrations in either the bile or plasma was seen upon the administration of 1.6 to 2.7 mg of copper (Fig. 1). On the other hand manganese concentrations increased markedly in both the bile and plasma after the administration of 3.9 to 11 mg of manganese (Fig. 2). In the bile, the maximum peak values showed a 26-fold increase with 3.9 mg, a 30-to 510-fold increase with 6.7 mg and a 90-to 1400-fold increase with 11mg compared with the values before the infusion. In the plasma, they were the highest at 11 AM just after the end of the infusion and 8 to 80 times as much as the values at 9 AM. Though not as remarkable as manganese, a considerable increase in zinc values was seen in both the bile and plasma upon administration of 3 to 6.5 mg (Fig. 3).

Furthermore, in Cases 1 to 5, the biliary excretion of these elements were examined both before and after the infusion to estimate the effect of the administration of them more precisely. The range of concentrations and excreted volumes of the three elements in the bile of Cases 1 to

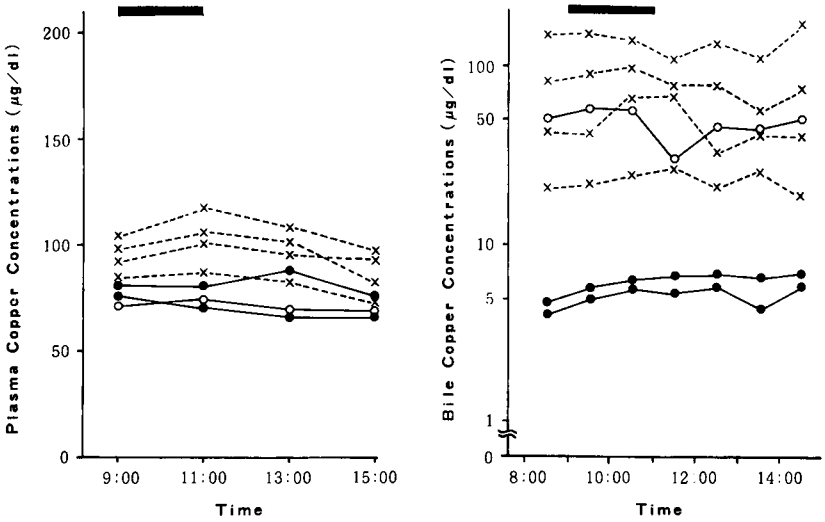


**Fig. 1.** The changes in copper concentrations in plasma and bile after the infusion of trace elements solutions. (○—○: 3A. of Trace Elements Solutions®. ×----×: 5A. of Trace Elements Solutions®. ●—●: 5A. of Trace Mineral Solutions®. ▬: during the infusion)

7 are shown in Table 4. No difference was seen in copper levels between the first and second day. Therefore the infusion of copper had little affect on its biliary excretion indicating that infused copper is excreted slowly. On the other hand, biliary excretion of manganese was increased after its infusion; though subsequently decreasing, the value on the second day was still higher than the pre-infusion value. In the subsequent change of the days before the infusion of Cases 1 to 3, zinc increased with time due to the circadian deviation. But the rate of the increment

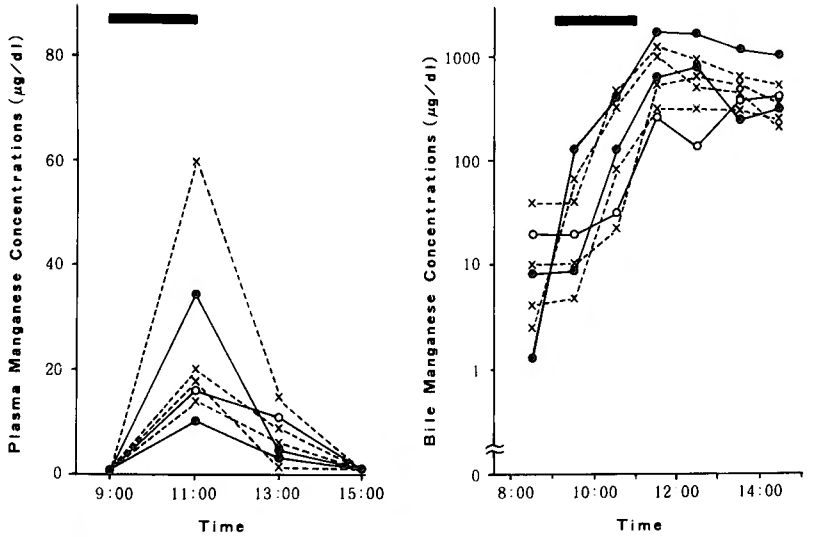


**Fig. 2.** The changes in manganese concentrations in plasma and bile after the infusion of trace elements solutions. (see Figure 1.)

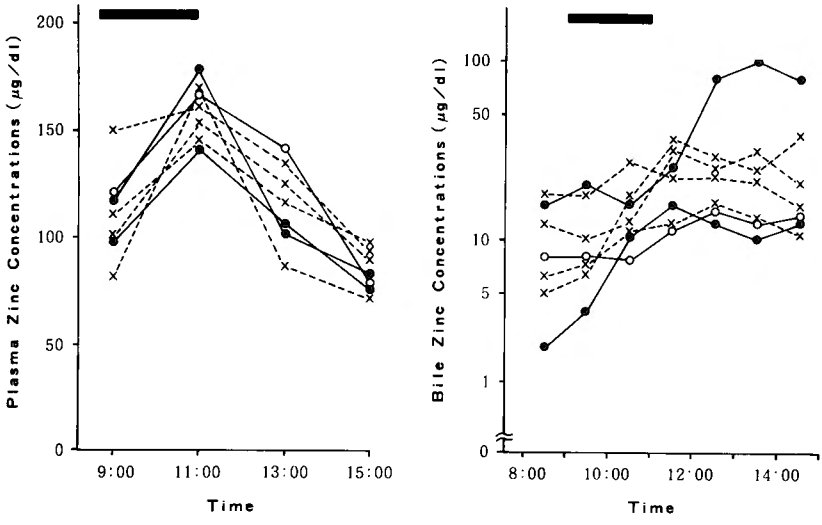


**Fig. 3.** The changes in zinc concentrations in plasma and bile after the infusion of trace elements solutions. (see Figure 1.)

on the day of infusion was greater than that before the infusion in all cases (Fig. 4). Although the difference of the excreted volumes between the first and second day were small, to some extent zinc excretion into the bile was induced by the infusion.

In addition, urinary excretion of trace elements was studied. Urine was collected from Cases 3 to 5 during a seven-hour period from 8 AM to 9 AM, 9 AM to 11 AM, 11 AM to 1 PM, and 1 PM to 3 PM (Table 5). With the infusion of trace elements solutions, leakage of a small

**Table 4.** Summary of the First Group.

| Case | Day | Bile Flow* (ml) | Copper | | | Manganese | | | Zinc | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Infused Volume (mg) | Range of Conc. (μg/dl) | Excreted* Volume (μg) | Infused Volume (mg) | Range of Conc. (μg/dl) | Excreted* Volume (μg) | Infused Volume (mg) | Range of Conc. (μg/dl) | Excreted* Volume (μg) |
| 1 | 1st | 133 | 0 | 14-32.4 | 27.0 | 0 | 3.6-5.7 | 6.2 | 0 | 13.2-28.9 | 28.4 |
| | 2nd | 118 | 2.7 | 18.8-28 | 27.0 | 6.7 | 2.7-352 | 226 | 5.0 | 10.3-32.8 | 31.3 |
| 2 | 1st | 206 | 0 | 3.6-5.6 | 9.0 | 0 | 6.1-8.0 | 15.0 | 0 | 19.6-56 | 61.1 |
| | 2nd | 190 | 1.6 | 4.0-5.8 | 9.8 | 11.0 | 9.2-840 | 775 | 6.5 | 17.4-103 | 99.0 |
| 3 | 1st | 122 | 0 | 4.4-6.4 | 7.0 | 0 | 1.0-2.0 | 1.7 | 0 | 2.1-12.2 | 3.7 |
| | 2nd | 128 | 1.6 | 4.5-6.8 | 6.8 | 11.0 | 1.3-1860 | 1200 | 6.5 | 2.6-16.5 | 12.4 |
| 4 | 1st | 113 | 2.7 | 5.6-96.8 | 83.4 | 6.7 | 42-1260 | 658 | 5.0 | 13.8-37.2 | 28.2 |
| | 2nd | 105 | 0 | 65-121 | 89.1 | 0 | 104-194 | 147 | 0 | 11-20 | 15.5 |
| 5 | 1st | 147 | 2.7 | 32.8-64.4 | 67.8 | 6.7 | 2.1-1080 | 719 | 5.0 | 5.1-32 | 26.3 |
| | 2nd | 147 | 0 | 28-55 | 64.5 | 0 | 76-125 | 149 | 0 | 12.8-24.4 | 27.8 |
| 6 | | 143 | 2.7 | 107-168 | 214 | 6.7 | 8.5-815 | 617 | 5.0 | 5.7-16.2 | 17.2 |
| 7 | | 124 | 1.6 | 29.5-57.5 | 55.9 | 3.9 | 15.5-408 | 37.9 | 3.0 | 18.4-38.4 | 28.9 |

* For seven hours from 8 AM to 3 PM.

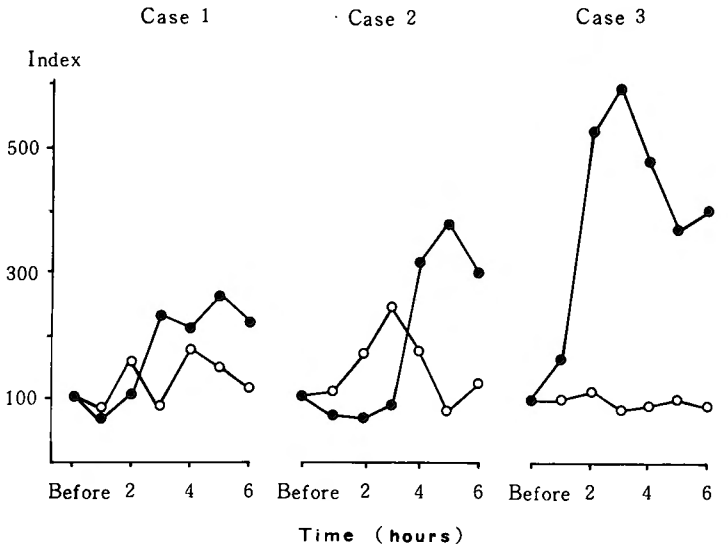


**Fig. 4.** The comparison of the changes in zinc concentrations in bile on the day of infusion of trace elements solutions with those of the previous day. The value of bile first obtained from 8 AM to 9 AM was taken as 100. Subsequent values were plotted as ratios of the value from the first collected bile sample.
(●—●: infused day, ○—○: previous day)

amount of manganese (Cases 3 and 5) and copper (Case 3) were detected in the urine. But in all cases, their concentrations in the urine were much less than in the bile, and the urine was not

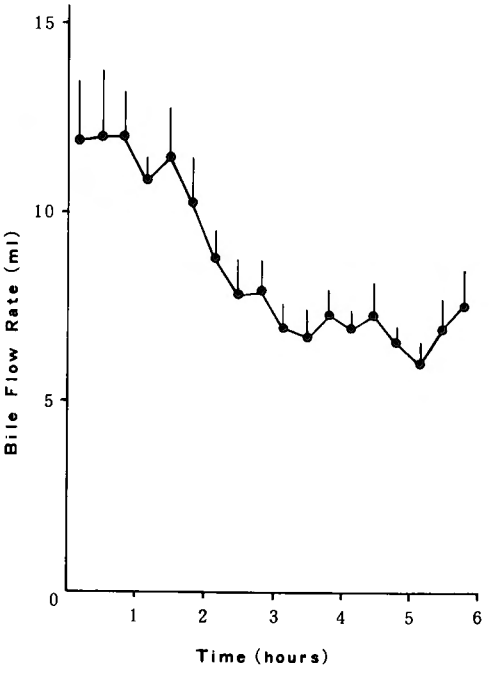


**Fig. 5.** Subsequent changes in bile flow rate collected 13 cases from the seven patients of group two. The flow rates were expressed as mean ±SEM every 20 minutes.

**Table 5.** Urinary Concentrations of Copper, Manganese and Zinc With or Without the Infusion of Trace Elements Solutions.

| Case | Day | Trace Elements | Collection Time 8AM–9AM | 9AM–11AM | 11AM–1PM | 1PM–3PM |
|---|---|---|---|---|---|---|
| 3 | 1st | Cu | * | * | * | * |
| | | Mn | 0.3 | * | * | * |
| | | Zn | 107 | 120 | 95.5 | 109 |
| | 2nd** | Cu | 0.8 | 1.3 | 1.9 | 0.9 |
| | | Mn | * | 12.7 | 15.0 | 2.7 |
| | | Zn | 120 | 105 | 130 | 115 |
| 4 | 1st** | Cu | * | * | * | * |
| | | Mn | 0.4 | * | * | * |
| | | Zn | 28.2 | 14.5 | 11.8 | 11.8 |
| | 2nd | Cu | * | * | * | * |
| | | Mn | * | * | * | * |
| | | Zn | 30.9 | 31.8 | 30 | 16.7 |
| 5 | 1st** | Cu | * | * | * | * |
| | | Mn | * | 1.7 | 3.8 | * |
| | | Zn | 40 | 40.2 | 59.8 | 30.7 |
| | 2nd | Cu | * | 1.9 | 0.9 | 0.5 |
| | | Mn | * | * | * | * |
| | | Zn | 149 | 156 | 101 | 154 |

(μg/dl)

* Not detectable under 0.1 μg/dl.
** With the infusion of trace elements solutions.

considered to be an important excretory pathway. On the other hand, in zinc, while the increase in the urine by the infusion was not seen, the concentrations in the urine were same or higher than in the bile, and the excreted volume for seven hours into the urine was two to ten times great than into the bile. Thus, unlike for the other two elements, urine is an important excretory pathway for zinc.

In second group, bile was collected from the complete biliary fistulas and various bile components were simultaneously determined. Total bile volume was 110 to 207 ml during a six-hour period from 9 AM to 3 PM in all cases. The flow rate gradually diminished with time probably because of the loss of bile acids[49] (Fig. 5). Biliary excretion patterns of copper and manganese without infusion were similar among the cases and highly characteristic (Figs. 6, 7). The subsequent changes of concentrations during the six-hour period on the first day for all seven cases were expressed as ratios of the first bile collection (index value, 100). Copper concentration was transiently reduced after one to two hours and then gradually increased. On the other hand, manganese concentrations dramatically decreased with eclampsia. The excretion patterns of them differed greatly. However, the zinc excretion pattern could not be characterized as it differed considerably from case to case.

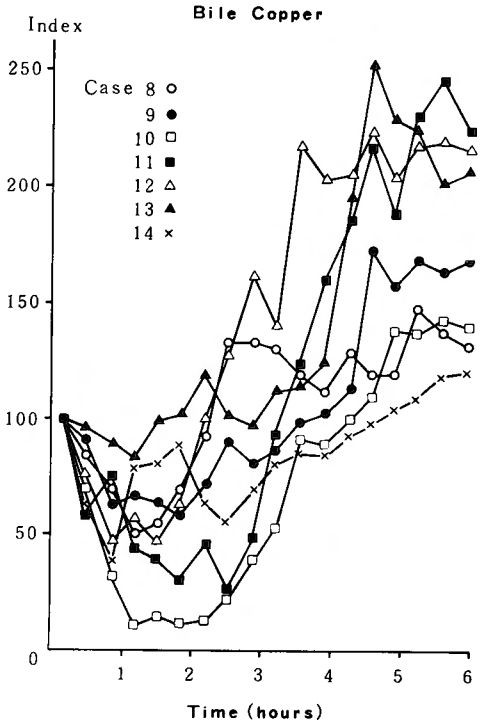


**Fig. 6.** Changes in bile copper values during six hours from 9 AM to 3 PM collected from complete biliary fistulas on the first day of seven cases. In each case, the value obtained from the first bile collection (from 9 AM to 9:20 AM) was given an index value of 100, and the subsequent values were plotted as ratios of the initial value.

In this group, trace elements were infused mixedly or singly as with the first group of patients and the biliary excretion was determined. Case 10 was infused with 1 mg of copper ($CuCl_2$-$5H_2O$) on the second day and the biliary excretion of copper during a six-hour period was compared with those of the first and third days. The pattern on the second day was not different from those of the other two days, however the concentration on that day was the highest among the three days (Fig. 8). This increase was probably not caused by the administration of copper, but by other events such as loss of bile, because an increase in concentration on the second day of the other cases without infusion was also seen (Table 6); namely, the loss of bile itself would bring about that phenomenon due to the adaptation. Thus, also when copper alone was infused, its concentration did not increased. On the other hand, the manganese concentration increased when infused both mixed and alone (Fig. 9). However, these concentrations rapidly decreased from the maximum peak and the excretion patterns were apparently different from those patients in the first group because the method of bile collection was different. From above result, it is apparent that the biliary excretion of copper is independent of that of manganese.

Next biliary excretion of other bile components were studied. The range of their concentrations in the first bile collection of seven cases were as follows: total bilirubin was 18.3 to 49.4 mg/dl; total bile acids, 31 to 80 $\mu$mol/ml; total protein, 85 to 273 mg/dl; magnesium, 2.2 to

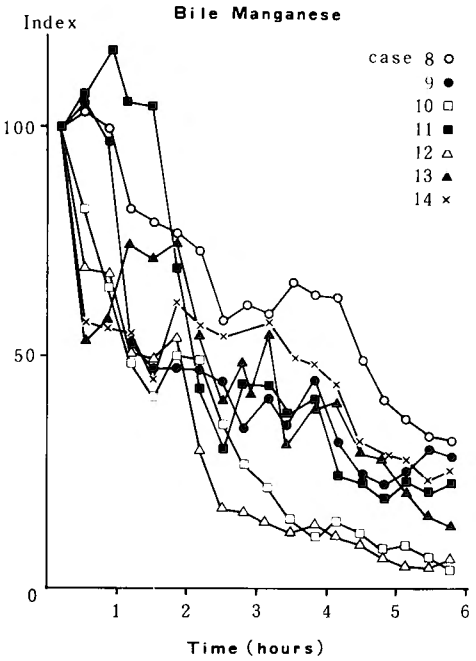


**Fig. 7.** Subsequent changes of bile manganese for six hours from 9AM to 3PM collected from complete biliary fistulas. See Figure 6.

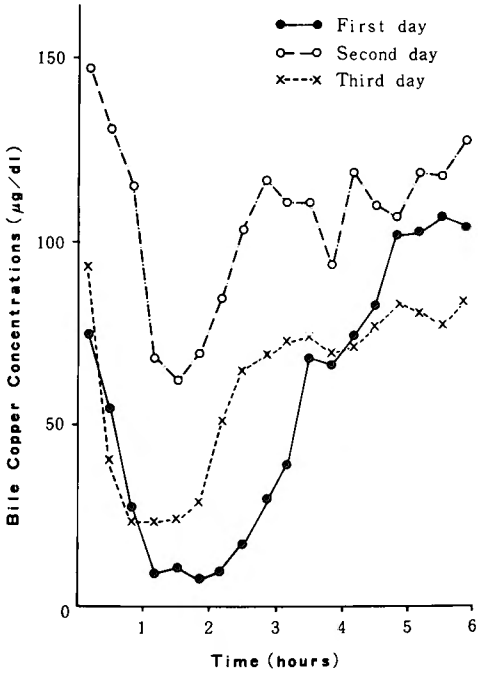


**Fig. 8.** Changes in copper concentrations in bile with or without the infusion of copper in Case 10.

**Table 6.** Summary of the Second Group.

| Case | Day | Bile Flow* (ml) | Copper | | Manganese | |
|---|---|---|---|---|---|---|
| | | | Range of Conc. (μg/dl) | Excreted Volume (μg) | Range of Conc. (μg/dl) | Excreted Volume (μg) |
| 8 | 1st | 122 | 63.7-185 | 160 | 5.2-16.6 | 13.5 |
| | 2nd | 123 | 106-200 | 180 | 4.0-13.5 | 10.3 |
| 9 | 1st | 127 | 6.0-17.8 | 12.4 | 2.3-11.6 | 8.1 |
| | 2nd | 125 | 20.5-62.7 | 48.2 | 1.0-9.3 | 6.2 |
| 10 | 1st | 185 | 7.1-107 | 73.9 | 0.1-2.5 | 4.9 |
| | 2nd[a)] | 172 | 61.9-147 | 146 | 0.2-1.8 | 0.9 |
| | 3rd | 188 | 23.7-94 | 109 | 0.1-3.4 | 2.3 |
| 11 | 1st | 114 | 3.0-40.5 | 17.7 | 11.7-91 | 46.1 |
| | 2nd[b)] | 110 | 23.6-103 | 57.8 | 20.6-391 | 239 |
| 12 | 1st | 184 | 4.1-24.3 | 27.6 | 0.3-8.1 | 6.9 |
| | 2nd[c)] | 160 | 6.2-73.9 | 52.8 | 2.7-186 | 115 |
| 13 | | 207 | 66.7-475 | 417 | 0.6-4.5 | 4.9 |
| 14 | | 177 | 4.9-10.6 | 13.8 | 2.0-6.4 | 6.6 |

* For six hours from 9 AM to 3 PM.
a) : With the infusion of Cu 1 mg.
b) : With the infusion of the Trace Elements Solution.
c) : With the infusion of Mn 1 mg.

3.7 mg/dl; and calcium, 5.9 to 7.2 mg/dl. These values were in a fairly narrow range compared with those of the trace elements. Biliary excretion patterns of these components during a six-hour period were similar in every case except for total protein, in which individual and diurnal

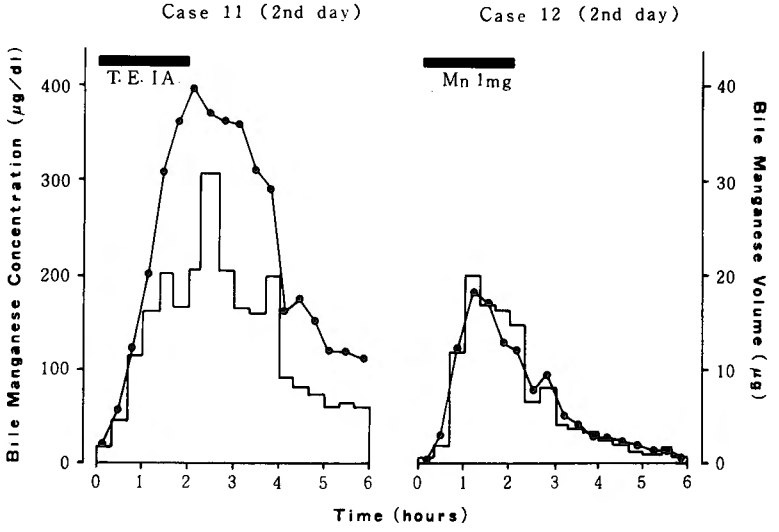


**Fig. 9.** The comparison of biliary excretion of manganese after the infusion of trace elements.
●—●: manganese concentration, bar graph: manganese volume for 20 minutes.

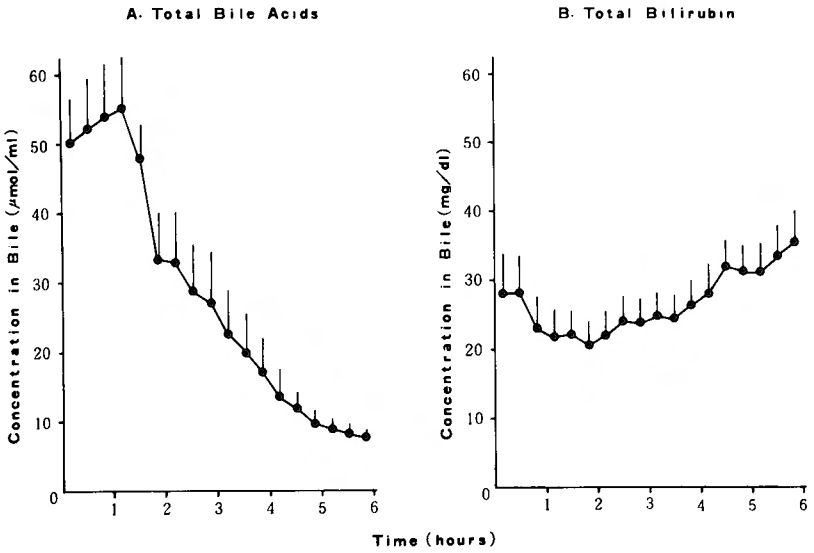


**Fig. 10.** Subsequent changes in total bilirubin and total bile acids in bile for six hours from 9 AM to 3 PM collected from complete biliary fistula in seven cases. The values were expressed as mean±SEM every 20 minutes.

deviations were too large to draw any conclusions. In the bile acids, a typical excretion pattern for the components was seen. As was also seen in previous experiments[13,28,49], the interruption of the enterohepatic circulation caused the concentration of the total bile acids to dramatically decrease with time in all cases (Fig. 10, panel A). In contrast, total bilirubin concentration

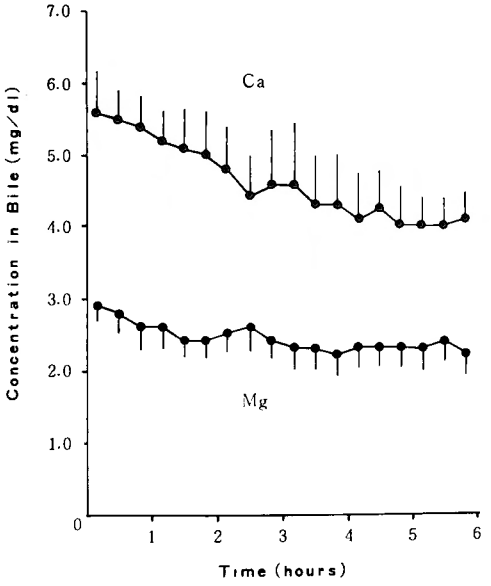


**Fig. 11.** Subsequent changes in calcium and magnesium in bile for six hours from 9 AM to 3 PM collected from seven cases. Calcium concentrations of five days and magnesium concentrations of 9 days were expressed as mean±SEM.

gradually increased after a transient reduction (Fig. 10, panel B). The concentrations of both magnesium and calcium in the bile was similar to those in plasma; these were different from other bile components and they decreased slightly with time. However, their decrease was not as marked as those of total bile acids (Fig. 11).

Comparing their excretion patterns with those of copper and manganese, interestingly, that of copper was similar to that of total bilirubin, and also that of manganese resembled that of total bile acids as shown in Case 12 (Fig. 12). The coefficients of correlation between copper and total bilirubin concentrations in 18 bile samples collected every 20 minutes for six hours in seven cases were from 0.713 to 0.942 ($P<0.001$) (Table 7). Between manganese and total bile acids, the coefficients of correlation were from 0.617 to 0.948 ($P<0.01$). This high correlation of trace elements to other organic substances is of great importance in understanding their biliary excretion, however, these findings were greatly affected by the methods of bile collection. Therefore, in addition to the above experiments, bile samples were collected by another procedure. Briefly, 1 ml of bile was obtained through a thin polypropylene tube inserted in choledochus via choledocal T tube, of which external crus was closed, every 30 minutes from 9 AM to 3 PM. Thus, while maintaining enterohepatic circulation, 13 bile samples were collected for six hours, and copper, manganese, total bilirubin and total bile acids levels were determined. The coeffici-

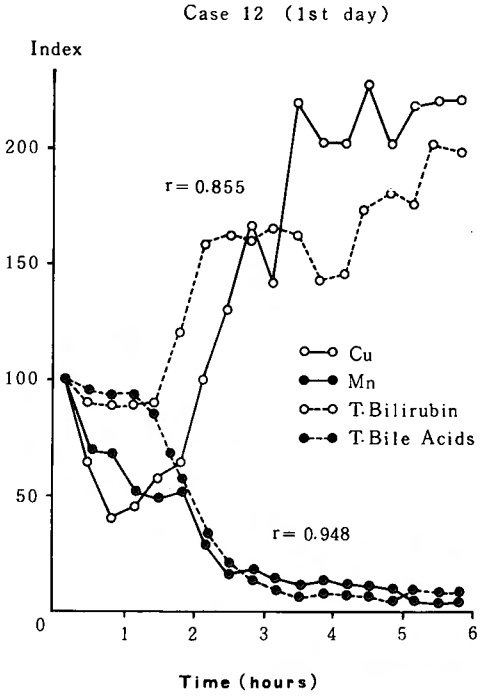


**Fig. 12.** The comparison of subsequent concentration changes in copper, manganese, total bilirubin and total bile acids in bile collected for six hours under complete biliary fistula on the first day of Case 12. The coefficient of correlation between copper and total bilirubin was 0.855, and it was 0.948 between manganese and total bile acids. Each substance was expressed as a ratio of the value of first collected bile.

**Table 7.** Correlation Coefficients Between Copper and Total Bilirubin, and Between Manganese and Total Bile Acids Concentration in the Bile.

| Case | Copper-Total Bilirubin | Manganese-Total Bile Acids |
|---|---|---|
| 8 | 0.797 | 0.834 |
| 9 | 0.726 | 0.637 |
| 10 | 0.785 | 0.793 |
| 11 | 0.713 | 0.702 |
| 12 | 0.855 | 0.948 |
| 13 | 0.942 | 0.617 |
| 14 | 0.899 | 0.714 |

ents of correlation between copper and total bilirubin in two cases were 0.783 and 0.837 ($P<0.01$). On the other hand, that of manganese and total bile acids were 0.583 and 0.109. Also from this method, high correlation was seen between copper and total bilirubin.

## Discussion

In the discussion of biliary excretion of trace elements, as a matter of course, it is very important to consider how the experiments were carried out because differences between species, the methods of bile collection and analytical techniques are important factors in the precise determination of trace elements[29]. The main purpose of our experiments was to understand the biliary excretion of trace elements in humans, and all samples were obtained from humans. However, there were various problems such as individual differences or many restrictions to avoid excessive burdening of the human body. Furthermore, the analytical technique used for the determination of total trace elements is not adequate for exogenously infused elements. Considering these limitation, the findings in this study were described with reference to previous reports.

The character of biliary excretion varied among the three elements and the dependence on bile of their excretion appeared to differ. Though bile is one of the most important pathway for both copper and manganese excretion as their concentrations in bile are much higher than those in the urine, for zinc, the excretion via pancreatic juice and the fluids of small intestine other than bile have been noted[53];from only one sample of pancreatic juice obtained from a fistula of the pancreatic duct, zinc concentration was 89.1 $\mu$g/dl (copper-1.9 $\mu$g/dl, manganese-0.3 $\mu$g/dl). In addition, in the first group, more zinc was found in the urine than in the bile, therefore bile is not as important for zinc excretion.

Copper is one of the most extensively studied elements for Wilson's disease. When 4.8 to 5.3 $\mu$g of labelled copper was injected in rats, about 25% of it was excreted in bile within 24 hours[38]. This large amount of biliary excretion was also reported by CIKRT[10]. Therefore, the remarkable increase of copper in the first group was expected, but not measured. According to MAHONEY, in the dog only about 8% of the infused copper was excreted into bile within 100 hours at dose levels of 0.1 to 0.2 mg per kg[31]. And in normal humans 24% to 40% of the injected

dose was excreted within 10 to 14 days[37]. Therefore, the rate of copper excretion varied among species. From the results of this study, it is more plausible that the administered copper is stored in body tissue and excreted slowly as seen in dogs[19].

It is well known that a large part of copper binds to celuroplasmin in plasma, but in bile the binding substances have not yet been found. Celuroplasmin in bile is too little and not a major carrier protein, and copper does not usually exist as an ion[1,18]; high molecular weight substance[18], taurochenodeoxycholate in micelle[30], low molecular weight protein[17] and amino-acids[15] have been proposed. According to LEWIS, in T tube bile of humans, a high correlation between copper and bile acids was found, but the method of bile collection was not clear. In the study described here, copper concentration were highly related to total bilirubin concentration, but not to bile acids. By using high pressure liquid chromatography, various fractions of bile acids including taurochenodeoxycholate were determined in bile of some cases[33], but no significant correlation between copper and these fractions was seen, thus, it is unlikely that copper is present in fractions of bile acids. Indeed, less biliary copper was reported to be absorbed than ionized copper when administered intraduodenally and the grade of the enterohepatic circulation is not so extensive[11,36]. From the result of this study, copper excretion into bile was fairly well regulated by a precise mechanism because there was no increase inspite of its administration, and rather, its excretion pattern was quite similar to the bilirubin excretion pattern. Therefore, though there is not clear evidence, it is possibly that copper excretion in bile is dependent on bilirubin and more copper might be excreted as a complex of the bilirubin components. This bilirubin-copper complex in bile was also suggested by another method[34]. However, though bilirubin-metal complex has been studied in vitro[23,54,55], the bilirubin-copper complex was not stable, and thus, this concept has not yet been confirmed. Further investigation is required.

Biliary excretion of manganese differs from that of copper in many points. A large proportion of injected manganese was excreted in rats[5,10,39], and also in this study rapid and drastic increase of its excreted concentration was seen by administration. The percentages of the increased manganese for six hours to the administered dose were 3.2% to 10.9% in Cases 1 to 3 and 10.9% to 14.4% in Cases 11 and 12. Though it is unknown whether the increase is caused by the infused manganese or the release of the endogenous one from this analytical technique, probably a large proportion of it resulted from exogenous manganese passed directly into bile from plasma because the increase was too rapid and extreme. Furthermore, manganese is certainly a typical substance involved in enterohepatic circulation as seen from the results of the depletion of its concentration and high correlation to bile acids in natural biliary excretion due to interruption of the enterohepatic circulation. As similar findings were reported in rats by tracer methods[5,11,39], in human also it may be true. However, it is difficult to account for the presence of manganese in the bile. In intraduodenally infused rats, biliary manganese was more easily absorbed than ionized manganese[11], and in the bile, free and bounded manganese have been reported in rats[5,50,51]. From results here, it is more likely that some of the manganese is excreted as free ions and some as bounded with the fractions of bile acids in natural excretion, and when overloaded, it would be excreted as free ions in bile from plasma due to direct passage.

For infused zinc, moderate increases in the bile were seen in the first group. However, the biliary excretion in the second group could not be characterized; this may be due to not only contamination error of the sample collection, but also to multiple pathways. Namely, zinc is excreted through many pathways and bile is not considered the main regulatory one of its excretion, thus, the biliary excretion of zinc is more difficult to understand.

*Brauer* has classified the bile components into three classes (A, B and C) from the concentration ratio of plasma to bile[8]. According to him, bilirubin, bile acids, copper and manganese belong to class B substances, as they are found more in the bile than in the plasma. On the other hand, magnesium and calcium are classified as class A; their levels in the bile are equal to those in the plasma; their concentrations in the bile were constant in many cases and the deviation during bile collections was little. Their biliary excretion is quite different from those of trace elements and they are transfered from the plasma to the bile by the body's regulating homeostasis.

On the other hand, the level of protein concentration in the bile was fairly lower than in the plasma, thus, it belongs to class C. It is important to characterize the biliary excretion of protein and clarify its role in the bile. Therefore, in addition to the determination of total protein, the fractions of protein in bile were simultaneously determined by the electrophoresis as is used in clinical examination, but both constant findings of biliary excretion of protein and a definite relationship with trace elements could not be obtained. Specific protein was present in the bile[12,20,58] and its excretion was too complex to be understood.

## Clinical application

In the surgical field the study of trace elements is of great concern in the administration of total parenteral nutrition and in the role of gallstone formation. These problems will be discussed in light of these present findings.

*Total Parenteral Nutrition*

Copper, manganese and zinc are essential trace elements and are necessary for the maintenance of health. Indeed as the use of total parenteral nutrition has increased so has the reports of zinc and/or copper deficiencies. Therefore, the dosage and method of administration are important problems. Though the required dosage of trace elements has almost been completely from the clinical experience during total parenteral nutrition or from the balance study of their elements in humans[3,22,24,25,43,44], there are no reports based on the fundamental experiments. From the results of this study, the recommended dosage and method of administration is proposed.

Copper: The administered copper was excreted slowly and seemed to be stored and deposited in body tissue; the total amount of copper in human body is only about 100 mg[53]. Therefore, it is dangerous to daily administer a large amount of dose. If the patients are in a stable condition, copper should be administered at a dose of about 1 mg daily or intermittently.

Manganese: The deficiency of this element has rarely been reported; in the human body there is only about 10–20 mg[53]. As it is apparently involved in enterohepatic circulation and the administration of only 1 mg of manganese induced considerable biliary excretion, it should not

be administered in a large daily dose; the administration of 0.5–1 mg of manganese twice a week should be adequate. As excess manganese was rapidly excreted there is little danger of toxicity, however, cholestasis with the infusion of bilirubin and manganese in rats has been reported[56]. Thus, the administration of it to the patients with jaundice should be avoided.

Zinc: Zinc is one of the elements in which deficiencies are most readily seen and the total volume in body is second that of iron. As it is present in many metalloenzymes[53], it plays an important role in metabolism. As zinc is excreted via the multiple pathways, it is difficult to determine the dose only from the biliary excretion. Considering the evidence of the increase in the bile after the infusion of 5 mg, it should be administered at a dose of 3-5 mg daily.

*Gallstone Formation*

Some kind of gallstones such as pigment stone, contain a considerable amount of inorganic ions of calcium, phosphorus and magnesium[9,46,47,57]. Infrared spectrometry[47], X-ray microprobe analizer[4,9] and atomic absorption spectrometry[57] have confirmed that trace elements of iron, copper and manganese are present in pigment stones to some extent. Of interest, in Japan, two types of pigment stones were found[21]. One is a dark brown stone (bilirubin stone) with bilirubin and calcium which is present in not only the gallbladder but also in another biliary tract. The other is a "black stone" containing a large amount of inorganic substances and black amorphous substances; it is mostly found in the gallbladder. In both of these types the bilirubin-calcium complex is considered to play an important role in the formation of these stones based on its high concentration[2,32,45]. On the other hand, the role of trace elements was considered to be minimal based only on the results of ion exchange[9].

However, as biliary excretion of trace elements were not sufficiently understood until now, the specificity of them was not taken into account in gallstone formation. As described here, bile is an important media for the excretion of trace elements and their excretory characters are very different from major ions such as calcium and magnesium. Especially, copper excretion was highly related to bilirubin and it seemed to be quite complicated.

In black stone, some investigators described that copper complex with bilirubin or bilirubin derivatives were important constituents[47,57]. Epidemiologically, pigment stones were frequently seen in liver cirrhosis[6], and in hemolytic anemia[16], suggesting the involvement of metabolic disorder in their formation[52]. Moreover, in Wilson's disease, pigment stones were also present in a high incidence[40]. This evidence suggests that the disorder of biliary excretion of copper, in addition to other bile components, plays an important role in the gallstone formation, especially in pigment stone.

**Ackowledgement**

This work was in part supported by Grant-in-Aid No. 00448253 for Scientific Research from the Japan Ministry of Education, Science and Culture.

## References


1) Aisen P, Morell AG, et al: Biliary excretion of celuroplasmin copper. Nature **203**: 873–874, 1964.
2) Allen B, Bernhoft R, et al: Sludge is calcium bilirubinate associated with bile stasis. Amer J Surg **141**: 51–56, 1981.
3) AMA Department of Foods and Nutrition: Guidelines for essential trace element preparations for parenteral use. A statement by an expert panel. JAMA **241**(19): 2051–2054, 1979.
4) Been JM, Bills PM, et al: Microstructure of gallstones. Gastroenterology **76**: 548–555, 1979.
5) Bertinchamps AJ, Miller ST, et al: Interdependence of routes excreting manganese. Am J Physio **211**(1): 217–224, 1966.
6) Bouchier IAD: Postmortem study of the frequency of gallstones in patients with cirrhosis of the liver. Gut **10**: 705–710, 1969.
7) Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**: 248–254, 1976.
8) Brauer RW: Mechanisms of bile secretion. JAMA **169**(13): 1462–1466, 1959.
9) Burnett W, Dwyer KR, et al: Black pigment or polybilirubinate gallstones: composition and formation. Ann Surg **193**(3): 331–333, 1981.
10) Cikrt M: Biliary excretion of $^{203}$Hg, $^{64}$Cu, $^{52}$Mn, and $^{210}$Pb in the rat. Brit J Industr Med **29**: 74–80, 1972.
11) Cikrt M: Enterohepatic circulation of $^{64}$Cu, $^{52}$Mn and $^{203}$Hg in rats. Arch Toxikol **31**: 51–59, 1973.
12) Dive Ch, Heremans JF: Nature and origin of the proteins of bile: I. A comparative analysis of serum and bile proteins in man. Eur J Clin Invest **4**(4): 235–239, 1974.
13) Dowling RH, Mack E, et al: Biliary lipid secretion and bile composition after acute and chronic interruption of the enterohepatic circulation in the rhesus monkey. IV. Primate biliary physiology. J Clin Invest **50**: 1917–1926, 1971.
14) Dowling RH, Mack E, et al: Experimental model for the study of the enterohepatic circulation of bile in rhesus monkeys. J Lab Clin Med **72**: 169–176, 1968.
15) Evans GW, Cornatzer WE: Biliary copper excretion in the rat. Proc Soc Exp Biol Med **136**: 719–721, 1971.
16) Fevery J, Verwilghen R, et al: Glucuronidation of bilirubin and the occurrence of pigment gallstones in patients with chronic haemolytic diseases. Eur J Clin Invest **10**(3): 219–226, 1980.
17) Frommer DJ: Biliary copper excretion in man and the rat. Digestion **15**: 390–396, 1977.
18) Gollan JL: Studies on the nature of complexes formed by copper with human alimentary secretions and their influence on copper absorption in the rat. Clin Sci Mol Med **49**(3): 237–245, 1975.
19) Goresky CA, Holmes TH, et al: The initial uptake of copper by the liver in the dog. Can J Physiol Pharmacol **46**: 771–784, 1968.
20) Hardwicke J, Rankin JG, et al: The loss of protein in human and canine hepatic bile. Clin Sci **26**: 509–517, 1964.
21) Hikasa Y, Nagase M, et al: Gallstone in western Japan-epidemiologic factors affecting the type and location of gallstones. Arch Jpn Chir **50**(2): 272–288, 1981.
22) Hull RL: Use of trace elements in intravenous hyperalimentation solutions. Amer J Hosp Pharm **31**: 759–761, 1974.
23) Hutchinson DW, Lohnson B, et al: Metal complexes of bilirubin in aprotic solvents. Biochem J **133**: 399–400, 1973.
24) Jacobson S, Wester PO: Balance study of twenty trace elements during total parenteral nutrition in man. Br J Nutr **37**: 107–126, 1977.
25) Jeejeebhoy KN, Langer B, et al: Total parenteral nutrition at home: studies in patients surviving 4 months to 5 years. Gastroenterology **71**(6): 943–953, 1976.
26) Karpel J, Peden V: Copper deficiency in long-term parenteral nutrition. J Pediatr **80**: 32–36, 1972.
27) Kay RG, Tasman-Jones C, et al: A syndrome of acute zinc deficiency during total parenteral alimentation in man. Ann Surg **183**: 331–340, 1976.
28) Klaassen CD: Bile flow and composition during bile acid depletion and administration. Can J Physiol Pharmacol **52**: 334–348, 1974.
29) Klaassen CD: Biliary excretion of metals. Drug Metab Rev **5**(2): 165–196, 1976.
30) Lewis KO: The nature of the copper complexes in bile and their relationship to the absorption and excretion of copper in normal subjects and in Wilson's disease. Gut **14**: 221–232, 1973.

31) Mahoney JP, Bush JA, et al: Studies on copper metabolism: XV. The excretion of copper by animals. J Lab Clin Med **46**: 702–708, 1955.
32) Maki T: Pathogenesis of calcium bilirubinate gallstone: role of E. coli, β-glucuronidase and coagulation by inorganic ions, polyelectrolytes and agitation. Ann Surg **164**: 90–100, 1966.
33) Maruyama K, Tanimura H, et al: Analysis of conjugated bile acids in bile by high pressure liquid chromatography. Clin Chim Acta **100**: 47–54, 1980.
34) Mccullars GM, O'Reilly S, et al: Pigment binding of copper in human bile. Clin Chim Acta **74**: 33–38, 1977.
35) Michaelsson M: Bilirubin determination in serum and urine. Studies on diazo methods and a new copper-azo pigment method. Scand J Clin Lab Invest **13**: (Suppl 56): 1–80, 1961.
36) Mistilis SP, Farrer PA: The absorption of biliary and non-biliary radiocopper in the rat. Scand J Gastroent **3**: 586–592, 1968.
37) O'Reilly S, Weber PM: Abnormalities of the physiology of copper in Wilson's disease. III. The excretion of copper. Arch Neurol **25**: 28–32, 1971.
38) Owen CA: Absorption and excretion of $Cu^{64}$-labeled copper by the rat. Am J Physiol **207**(6): 1203–1206, 1964.
39) Papavasiliou PS, Miller ST, et al: Role of liver in regulating distribution and excretion of manganese. Am J Physiol **211**(1): 211–216, 1966.
40) Rosenfield N, Grand RJ, et al: Cholelithiasis and Wilson disease. J Pediatr **92**(2): 210–213, 1978.
41) Schwartz MK: Role of trace elements in cancer. Cancer Res **35**: 3481–3487, 1975.
42) Sekiya T, Tanimura H, et al: Simplified determination of copper, zinc and manganese in plasma and bile by flameless atomic absorption spectrometry. Arch Jpn Chir **50**(6): 729–739, 1981.
43) Shils ME: Minerals in total parenteral nutrition. Drug Intell Clin Pharm **6**: 385–393, 1972.
44) Solomons NW, Layden TJ, et al: Plasma trace metals during total parenteral alimentation. Gastroenterology **70**: 1022–1025, 1976.
45) Soloway RD, Trotman BW, et al: Pigment gallstones. Gastroenterology **72**: 167–182, 1977.
46) Sutor DJ, Wooley SE: The nature and incidence of gallstones containing calcium. Gut **14**: 215–220, 1973.
47) Suzuki N, Nakamura Y, et al: On the metal elements in pure pigment gallstones. Tohoku J Exp. Med **116**: 233–240, 1975.
48) Talalay P: Enzymic analysis of steroid hormones. Meth Biochem Anal **8**: 119–143, 1960.
49) Thurebore E: Human hepatic bile: Composition changes due to altered hepatic circulation. Acta Chir Scandinav Suppl **303**: 1–63, 1963.
50) Tichy M, Cikrt M, et al: Manganese binding in rat bile. Arch Toxikol **30**: 227–236, 1972.
51) Tichy M, Cikrt M: Manganese transfer into the bile in rats. Arch Toxikol **29**: 51–58, 1972.
52) Tritapepe R, di Padova C, et al: Are pigmented gall stones caused by a "metabolic" liver defect?. Br Med J **22**: 832, 1980.
53) Underwood EJ: Trace elements in human and animal nutrition. New York, Academic Press Inc 4th ed 1977.
54) Van Norman JD, Humans MM: Bilirubin-metal ion complexes. Anal Chem **46**(7): 926–929, 1974.
55) Velapoldi RA, Menis O: Formation and stabilities of free bilirubin and bilirubin complexes with transition and rare-earth elements. Clin Chem **17**(12): 1165–1170, 1971.
56) Witzleben CL, Pitlick P, et al: Acute manganese overload: a new experimental model of intrahepatic cholestasis. Amer J Path **53**(3): 409–423, 1968.
57) Wosiewitz U, Schroebler S: On the chemistry of 'black' pigment stones from the gallbladder. Clin Chim Acta **89**: 1–12, 1978.
58) Yoon DS, Shim BS, et al: Bile-specific protein components in human hepatic bile. J Lab Clin Med **67**: 640–649, 1966.

和文抄録

# 銅，マンガン，亜鉛の胆汁排泄に関する臨床的研究

京都大学医学部外科学教室第2講座（主任：日笠頼則教授）
関谷　司，谷村　弘，日笠頼則

銅，マンガン，亜鉛といった微量元素は，生体中に極めて微量にしか存在しないため，特定の疾患以外，特に注目されずに現在にいたっている．しかし，それらは微量であっても，生体中で甚だ重要な役割をはたしていることが，栄養学，生理学の発達と共に次第に解明されつつある．一方，外科領域においても，完全静脈栄養の普及と共に，銅・亜鉛欠乏症がしばしば報告されるにいたり，その必要投与量が問題となってきた．また，黒色石等の胆石には，銅やその他の微量元素がかなり含有されていると指摘され，胆石形成にはたすこれら微量元素の役割を解明することが，重要な課題である．

銅，マンガンは，他の主要電質解質イオンと異なり，大部分が胆汁を介して糞便中に排泄されるという，極めて特異な排泄経路を有する．しかしながら，胆汁採取の困難さ，試料採取時の汚染の問題，適当な微量元素分析技術の欠如等の諸問題が未解決であったため，今日まで人における微量元素の胆汁排泄は，殆ど解明されていない．特に，腸肝循環という非常に複雑な問題が介在するため，その研究を一層困難にしてきた．

著者らは，これらの問題点を鑑み，通常のTチューブの他に，特殊なバルーン付Tチューブを作製し，それらを挿入した胆石症例を対象とし，微量元素添加液を投与しながら経時的に胆汁および血漿，尿を採取し，新しく開発したフレームレス原子吸光法を用いて，銅，マンガン，亜鉛を測定し，その排泄について検討を加えた．さらに，腸肝循環遮断中に採取した胆汁中の，総ビリルビン，胆汁酸，総蛋白，マグネシウム，カルシウム等の諸成分をも同時に測定し，それらと比較することにより，微量元素の胆汁排泄の特徴を究明し，完全静脈栄養時の微量元素投与量や，胆石形成における微量元素の役割について考察を加えた．その結果，

1) 銅は，2.7mgまでの静脈内投与では，胆汁中，血漿中共に濃度上昇は認められず，また尿からの排出も殆どみられなかった．一方，腸肝循環遮断時の排泄パターンは，時間と共にその濃度が漸次上昇し，総ビリルビンの排泄パターンと極めて類似していた．

2) マンガンは，1mgの静脈内投与ですら，胆汁中，血漿中共に著明な濃度上昇を示し，一部尿中へも排泄された．また腸肝循環遮断により急速な胆汁中濃度の減少を示し，総胆汁酸とも高い相関性を有していた．この事実より，マンガンの胆汁排泄にしめる腸肝循環の役割が，極めて重要な因子であることを明らかにした．

3) 亜鉛は，マンガン程著明ではないか，5mg投与により，胆汁中，血漿中共に濃度上昇が認められた．また，膵液および尿中の亜鉛は胆汁中亜鉛よりも濃度が高く，胆汁以外にも膵液や尿など種々の経路を介して排泄されると考えられる．

4) 胆汁中マグネシウム，カルシウムは，いずれの症例においても血漿中と殆ど同濃度であり，またその経時的変動もわずかであった．

以上の臨床的事実から，銅は排泄が遅く，組織内沈着の可能性があり，完全静脈栄養時に大量かつ連日投与することは危険であるといえる．また，総ビリルビンとの高い相関性より，胆汁中における銅は，ビリルビンの排泄と強い関係があると示唆され，胆石形成に際し重要な役割をはたしている可能性がある．

マンガンは，少量の静脈内投与でも急速に胆汁中に排泄され，また腸肝循環を行っていることも証明され，完全静脈栄養時には，少量の間歇的投与で十分であるといえる．

亜鉛の投与量については，胆汁以外にもいくつかの排泄路があるため，明解な結論は得られなかった．

従って，微量元素液として画一的な三者混合同時投与などは，極めて危険であり，各微量元素の排泄機序を考慮しながら，血漿中銅，マンガンおよび亜鉛濃度を測定し，その投与量を決定することが必要であることを指摘したい．